

AMENDMENTS

Amendments to the Specification:

- I. Please replace the title of the application, located on page 1, line 1, with the following substitute title:

**METHOD FOR MODULATING CELL PROLIFERATION IN
THE SEED COAT AND/OR INTEGUMENT Seeds**

- II. Please replace the paragraph located on page 21, lines 1–2, with the following substitute paragraph:

Figure 6 is an alignment of wild-type MNT (SEQ ID NO: 55) and mutant mnt-I cDNAs (SEQ ID NO: 5) from translational start to stop;

- III. Please replace the paragraph located on page 21, line 3, with the following substitute paragraph:

Figure 7 is an alignment of wild-type MNT (SEQ ID NO: 3) and mutant mnt-1 (SEQ ID NO: 6) predicted proteins;

- IV. Please replace the paragraph located on page 21, lines 4–5, with the following substitute paragraph:

Figure 8 is an alignment of *Arabidopsis thaliana* MNT cDNA (SEQ ID NO: 55) with its orthologue in *Brassica napus*, BnARF2 (SEQ ID NO: 9);

V. Please replace the paragraph located on page 21, lines 6–8, with the following substitute paragraph:

Figure 9 is an alignment of *Arabidopsis thaliana* MNT predicted protein (SEQ ID NO: 3) with its orthologues in *Brassica napus* (oilseed rape) (BnARF2) (SEQ ID NO: 10) and *Oryza sativa* (rice) (OsARF2) (SEQ ID NO: 61);

VI. Please replace the paragraph located on page 23, lines 19-25, with the following replacement paragraph:

The following vectors are used in the examples:

pGEMT (Promega, Southampton, UK)

BJ36, BJ40, BJ60 (gift of Bart Janssen, Horticultural & Food Research Institute of New Zealand)

pART7 (Gleave, 1992)

pFGC5941 (Cambia, Canberra, Australia; ChromDB, <http://www.chromdb.org/plasmids>)

VII. Please replace the paragraph located on page 33, lines 1-24, with the following replacement paragraph:

We mapped the MNT locus to a 60.9 kb region of chromosome 5 that was annotated by The Arabidopsis Information Resource (TAIR) (<http://www.arabidopsis.org>) to contain 17 genes. T-DNA insertion lines with insertions in these genes generated by The Salk Institute Genome Analysis Laboratory (SIGnAL (Alonso et al., 2003) (<http://signal.salk.edu>)) were obtained from the Nottingham Arabidopsis Stock Centre (NASC) (<http://nase.nott.ac.uk>). Salk line no. 108995 (NASC stock no. N608995), with an insertion in the coding region of the AUXIN RESPONSE FACTOR 2 (ARF2) gene, included a plant homozygous for the insertion with a similar phenotype to *mnt-1* mutants, including closed flowers and large seeds (FIG. 5A-C). Genotypic scoring of segregants from the Salk 108995 family, including one heterozygote and the homozygote, is shown in FIG. 5D. Specifically in FIG. 5D Top: Scoring for presence of an

insertion in the ARF2 gene. Primers used were 5' TGG TTC ACG TAG TGG GCC ATC G 3' (SEQ ID NO: 62), and 5' GAG TGG GTG GAG TGT GTT TG 3' (SEQ ID NO: 63). Lanes M and O show presence of the insertion. Bottom: Scoring for homozygous insertion mutants. Primers used were 5' GAG TGG GTG GAG TGT GTT TG 3' (SEQ ID NO: 63) and 5' AGT TGG TTT TCG TTT GAG CAT 3' (SEQ ID NO: 64). PCR conditions are set so that the gene will only amplify if there is no insertion: therefore PCR products will be amplified from DNA extracted from wild-type plants and also those hemizygous for the insertion, but not homozygous plants. Lane M shows no amplification, indicating this plant is homozygous for the insertion. An allelism test was conducted by crossing a seed parent homozygous for the mnt-1 mutation with the Salk 108995 homozygote as pollen parent. F1 progeny were hemizygous for the insertion (FIG. 5E) and had the mnt-1 mutant phenotype (FIG. 5F), confirming that MNT is the ARF2 gene.

VIII. Please replace the paragraph located on page 45, lines 1-19, with the following replacement paragraph:

Wild-type plants transformed with the 35S::MNT cassette described in Example 8a, b have the mnt mutant phenotype, including closed flowers for most of the plant's life cycle (FIG. 17B, top), and large seeds. Seeds from three independently transformed lines, along with wild-type plants grown under the same conditions, are shown in FIG. 17B, middle. The overall mean weight for these three lines was 25.5 µg, compared with 15.0 µg for the wild-type control. Expression of MNT/ARF2 was assayed in transformed and wild-type plants by semiquantitative RT-PCR (FIG. 17B, bottom) using multiplex RT-PCR with primers RTARF2-F (5'-GAGTGGGTGGAGTGTGTTTG-3') (SEQ ID NO: 63) and RTARF2-R (5'-AGTTGGTTTTTCGTTTGAGCAT-3') (SEQ ID NO: 64), and control primers to the GAPC gene, GAPC-F (5'-CACTTGAAGGGTGGTGCCAAG-3') (SEQ ID NO: 65) and GAPC-R (5'-CCTGTTGTCGCCAACGAAGTC-3') (SEQ ID NO: 66). PCR was initiated with RTARF2 primers and run for 4 cycles at an annealing temperature of 55°C, extension time 2 min. GAPC primers were added to each reaction mix and the reaction was run for an additional 22 cycles.

This showed that plants transformed with the 35S::MNT cassette did not have lower levels of MNT expression than wild-type plants; therefore the mutant phenotype was not due to cosuppression. Therefore constitutive expression of the MNT gene (such as achieved under control of the 35S promoter) provides a further method for producing large seeds.